

What is claimed is:

1. A method for reducing non-specific amplification of DNA in a polymerase chain reaction comprising the steps of:
  - (a) providing a sample comprising a target DNA sequence of interest;
  - (b) contacting said sample with at least one enzyme having nucleic acid polymerase activity; and
  - (c) incubating said sample with said enzyme for a time and under conditions sufficient to amplify said target DNA sequence, forming amplified target DNA sequence;wherein said incubation is performed in the presence of an amount of sorbitol effective to reduce said non-specific amplification relative to the amount of non-specific amplification observed in the absence of sorbitol.
2. The method of claim 1 further comprising an effective amount of DMSO to increase the yield of said amplified target DNA sequence relative to the amount of said amplified target DNA sequence observed in the absence of DMSO.
3. The method of claim 1 wherein the sorbitol is present in an amount of 0.05 M to 3 M.
4. The method of claim 1 wherein the sorbitol is present in an amount of 0.1 M to 2 M.
5. The method of claim 1 wherein the sorbitol is present in an amount of 0.2 M to 1 M.
6. The method of claim 1 wherein the sorbitol is present in an amount of 0.25 M to 0.5 M.
7. The method of claim 2 wherein said DMSO is present in an amount of 0.5% to 8.0%.
8. The method of claim 2 wherein said DMSO is present in an amount of 1.0% to 6.0%.
9. The method of claim 2 wherein said DMSO is present in an amount of 2.0% to 5.0%.

10. The method of claim 2 wherein said DMSO is present in an amount of 3.0% to 4.0%.
11. The method of claim 2 wherein said DMSO is present in an amount of 1.25% and said sorbitol is present in an amount of 0.15 M.
12. The method of claim 2 wherein said non-specific amplification is reduced to less than 99% or more of the amount of non-specific amplification obtained in the absence of said sorbitol and DMSO.
13. The method of claim 2 wherein said non-specific amplification is reduced to less than 90% of the amount of non-specific amplification obtained in the absence of said sorbitol and DMSO.
14. The method of claim 2 wherein said non-specific amplification is reduced to less than 80% of the amount of non-specific amplification obtained in the absence of said sorbitol and DMSO.
15. The method of claim 2 wherein said non-specific amplification is reduced to less than 70% of the amount of non-specific amplification obtained in the absence of said sorbitol and DMSO.
16. The method of claim 2 wherein said non-specific amplification is reduced to less than 60% of the amount of non-specific amplification obtained in the absence of said sorbitol and DMSO.
17. The method of claim 2 wherein said non-specific amplification is reduced to less than 50% of the amount of non-specific amplification obtained in the absence of said sorbitol and DMSO.

18. The method of claim 2 wherein said non-specific amplification is reduced to less than 40% of the amount of non-specific amplification obtained in the absence of said sorbitol and DMSO.
19. The method of claim 2 wherein said non-specific amplification is reduced to less than 30% of the amount of non-specific amplification obtained in the absence of said sorbitol and DMSO.
20. The method of claim 2 wherein said amplified target sequence represents at least 50-70% of said total amplified product.
21. The method of claim 2 wherein said amplified target sequence represents at least 70-90% of said total amplified product.
22. The method of claim 2 wherein said amplified target sequence represents at least 90% of said total amplified product.
23. The method of claim 1 wherein said DNA encodes ribosomal RNA.
24. The method of claim 2 wherein said DNA encodes ribosomal RNA.
25. The method of claim 1 wherein said amplification comprises contacting said nucleobase sequence with an enzyme having a polymerase activity.
26. The method of claim 1 wherein the enzyme having polymerase activity is selected from a DNA polymerase from *Thermus* species, *Bacillus* species, *Thermococcus* species, *Thermotoga* species, and *Pyrococcus* species.
27. The method of claim 1 wherein the enzyme having polymerase activity is selected from

the group consisting of AmpliTaq Gold® DNA polymerase; AmpliTaq® DNA Polymerase; AmpliTaq® DNA Polymerase, Stoffel fragment; *rTth* DNA Polymerase; *rTth* DNA Polymerase XL; *Tne*, Bst DNA polymerase large fragment from *Bacillus stearothermophilus*; Vent and Vent Exo- from *Thermococcus litoralis*; *Tma* from *Thermotoga maritima*; Deep Vent and Deep Vent Exo- and *Pfu* from *Pyrococcus*; and mutants, variants and derivatives thereof.

28. The method of claim 2 wherein said amplification comprises contacting said nucleobase sequence with an enzyme having a polymerase activity.

29. The method of claim 2 wherein the enzyme having polymerase activity is selected from a DNA polymerase from *Thermus* species, *Bacillus* species, *Thermococcus* species, *Thermotoga* species, and *Pyrococcus* species.

30. The method of claim 2 wherein the enzyme having polymerase activity is selected from the group consisting of AmpliTaq Gold® DNA polymerase; AmpliTaq® DNA Polymerase; AmpliTaq® DNA Polymerase, Stoffel fragment; *rTth* DNA Polymerase; *rTth* DNA Polymerase XL; *Tne*, Bst DNA polymerase large fragment from *Bacillus stearothermophilus*; Vent and Vent Exo- from *Thermococcus litoralis*; *Tma* from *Thermotoga maritima*; Deep Vent and Deep Vent Exo- and *Pfu* from *Pyrococcus*; and mutants, variants and derivatives thereof.

31. A method of amplifying ribosomal DNA in a polymerase chain reaction comprising the steps of:

- (a) providing a sample comprising a ribosomal DNA target sequence of interest; and
- (b) amplifying at least one nucleobase sequence of said ribosomal DNA to form amplified ribosomal DNA in a mixture of total amplified product;

wherein said amplification is performed in the presence of a sufficient amount of sorbitol and DMSO to reduce non-specific amplification relative to the amount of non-specific

amplification observed in the absence of said sorbitol and said DMSO.

32. The method of claim 31 wherein said sorbitol is present in an amount of 0.05 M to 3 M.
33. The method of claim 31 wherein the sorbitol is present in an amount of 0.1 M to 2 M.
34. The method of claim 31 wherein the sorbitol is present in an amount of 0.2 M to 1 M.
35. The method of claim 31 wherein the sorbitol is present in an amount of 0.25M to 0.5 M.
36. The method of claim 31 wherein said DMSO is present in an amount of 0.5% to 8.0%.
37. The method of claim 31 wherein said DMSO is present in an amount of 1.0% to 6.0%.
38. The method of claim 31 wherein said DMSO is present in an amount of 2.0% to 5.0%.
39. The method of claim 31 wherein said DMSO is present in an amount of 3.0% to 4.0%.
40. The method of claim 31 wherein said DMSO is present in an amount of 1.25% and said sorbitol is present in an amount of 0.15 M.
41. The method of claim 31 wherein said amplification comprises contacting said nucleobase sequence with an enzyme having a polymerase activity.
42. The method of claim 31 wherein the enzyme having polymerase activity is selected from a DNA polymerase from *Thermus* species, *Bacillus* species, *Thermococcus* species, *Thermotoga* species, and *Pyrococcus* species.
43. The method of claim 31 wherein the enzyme having polymerase activity is selected from

the group consisting of AmpliTaq Gold® DNA polymerase; AmpliTaq® DNA Polymerase; AmpliTaq® DNA Polymerase, Stoffel fragment; *rTth* DNA Polymerase; *rTth* DNA Polymerase XL; *Tne*, Bst DNA polymerase large fragment from *Bacillus stearothermophilus*; Vent and Vent Exo- from *Thermococcus litoralis*; *Tma* from *Thermotoga maritima*; Deep Vent and Deep Vent Exo- and *Pfu* from *Pyrococcus*; and mutants, variants and derivatives thereof.

44. A composition comprising:

- (a) a nucleic acid molecule comprising a sequence encoding a ribosomal DNA;
- (b) at least two primers having a sequence that is complementary to a portion of said nucleic acid sequence adjacent to said ribosomal DNA;
- (c) at least one enzyme having nucleic acid polymerase activity; and
- (d) sorbitol.

45. The composition of claim 44 further comprising DMSO.

46. A kit for the amplification of DNA comprising, in one or more containers: an agent comprising a polymerase activity, a plurality of deoxynucleotide triphosphates; and sorbitol, wherein said sorbitol is provided in an amount effective to reduce non-specific amplification relative to the amount of non-specific amplification observed in the absence of sorbitol.

47. The kit of claim 46 further comprising DMSO.

48. The kit of claim 46 wherein the enzyme having polymerase activity is selected from a DNA polymerase from *Thermus* species, *Bacillus* species, *Thermococcus* species, *Thermotoga* species, and *Pyrococcus* species.

49. The kit of claim 46 wherein the enzyme having polymerase activity is selected from the group consisting of AmpliTaq Gold® DNA polymerase; AmpliTaq® DNA Polymerase; AmpliTaq® DNA Polymerase, Stoffel fragment; *rTth* DNA Polymerase; *rTth* DNA Polymerase XL; *Tne*, Bst

DNA polymerase large fragment from *Bacillus stearothermophilus*; Vent and Vent Exo- from *Thermococcus litoralis*; *Tma* from *Thermotoga maritima*; Deep Vent and Deep Vent Exo- and *Pfu* from *Pyrococcus*; and mutants, variants and derivatives thereof.

50. The kit of claim 47 wherein the enzyme having polymerase activity is selected from a DNA polymerase from *Thermus* species, *Bacillus* species, *Thermococcus* species, *Thermotoga* species, and *Pyrococcus* species.

51. The kit of claim 47 wherein the enzyme having polymerase activity is selected from the group consisting of AmpliTaq Gold® DNA polymerase; AmpliTaq® DNA Polymerase; AmpliTaq® DNA Polymerase, Stoffel fragment; *rTth* DNA Polymerase; *rTth* DNA Polymerase XL; *Tne*, Bst DNA polymerase large fragment from *Bacillus stearothermophilus*; Vent and Vent Exo- from *Thermococcus litoralis*; *Tma* from *Thermotoga maritima*; Deep Vent and Deep Vent Exo- and *Pfu* from *Pyrococcus*; and mutants, variants and derivatives thereof.

52. A method of detecting bacteria in a sample comprising providing a sample comprising nucleic acid, said nucleic acid comprising at least one ribosomal DNA sequence; and amplifying at least one nucleobase sequence of said nucleic acid, thereby forming an amplified product, wherein said amplification is performed in the presence of an amount of sorbitol effective in reducing non-specific amplification relative to the amount of non-specific amplification observed in the absence of sorbitol.

53. The method of claim 52 wherein said amplification step further comprises an amount of DMSO effective to reduce non-specific amplification relative to the amount of non-specific amplification observed in the absence of DMSO.

54. The method of claim 52 wherein said amplification step comprises contacting said nucleic acid sequence with an enzyme having a polymerase activity.

55. The method of claim 54 wherein the enzyme having polymerase activity is selected from a DNA polymerase from *Thermus* species, *Bacillus* species, *Thermococcus* species, *Thermotoga* species, and *Pyrococcus* species.

56. The method of claim 54 wherein the enzyme having polymerase activity is selected from the group consisting of AmpliTaq Gold® DNA polymerase; AmpliTaq® DNA Polymerase; AmpliTaq® DNA Polymerase, Stoffel fragment; *rTth* DNA Polymerase; *rTth* DNA Polymerase XL; *Tne*, Bst DNA polymerase large fragment from *Bacillus stearothermophilus*; Vent and Vent Exo- from *Thermococcus litoralis*; *Tma* from *Thermotoga maritima*; Deep Vent and Deep Vent Exo- and *Pfu* from *Pyrococcus*; and mutants, variants and derivatives thereof.

57. The method of claim 53 wherein said amplification step comprises contacting said nucleic acid sequence with an enzyme having a polymerase activity.

58. The method of claim 57 wherein the enzyme having polymerase activity is selected from a DNA polymerase from *Thermus* species, *Bacillus* species, *Thermococcus* species, *Thermotoga* species, and *Pyrococcus* species.

59. The method of claim 57 wherein the enzyme having polymerase activity is selected from the group consisting of AmpliTaq Gold® DNA polymerase; AmpliTaq® DNA Polymerase; AmpliTaq® DNA Polymerase, Stoffel fragment; *rTth* DNA Polymerase; *rTth* DNA Polymerase XL; *Tne*, Bst DNA polymerase large fragment from *Bacillus stearothermophilus*; Vent and Vent Exo- from *Thermococcus litoralis*; *Tma* from *Thermotoga maritima*; Deep Vent and Deep Vent Exo- and *Pfu* from *Pyrococcus*; and mutants, variants and derivatives thereof.

60. The method of claim 52 further comprising determining the nucleic acid sequence of said amplified product and comparing said nucleic sequence of said amplified product with known bacterial ribosomal DNA sequences.



61. The method of claim 53 further comprising determining the nucleic acid sequence of said amplified product and comparing said nucleic sequence of said amplified product with known bacterial ribosomal DNA sequences.
62. The method of claim 60 wherein said amplified product is purified prior to determining said nucleic acid sequence of said amplified product.
63. The method of claim 61 wherein said amplified product is purified prior to determining said nucleic acid sequence of said amplified product.
64. The method of claim 55 wherein said sample is a clinical sample selected from the group consisting of blood, urine, cerebrospinal fluid, serum, saliva, mucus, skin scraping, gastric secretions and stool.
65. The method of claim 53 wherein said sample is a clinical sample selected from the group consisting of blood, urine, cerebrospinal fluid, serum, saliva, mucus, skin, gastric secretions and stool.